

U. Arup, M. Bertrand, P. Navarro-Rosinés, P. L. Nimis, C. Roux & U. Söchting

## Taxonomy and nomenclature of a much misunderstood Mediterranean lichen, *Caloplaca subochracea* auct. (Lichenized *Ascomycota*, *Teloschistaceae*)

### Abstract

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This paper clarifies the taxonomy and nomenclature of a characteristic calcicolous lichen, formerly called *Caloplaca subochracea* auct., which is widespread in coastal areas of the Mediterranean region. In spite of being easily recognizable, this lichen was often misunderstood, and has a very complicated nomenclatural history. The analysis of the type material of *Calloplisma marmoratum* Bagl., described from Sardinia in 1879, revealed that it is identical to *Caloplaca subochracea* auct., and has nothing to do with the lichen currently called *Xanthocarpia marmorata* (Bagl.) Frödén, Arup & Söchting. The results of molecular analysis showed that *Caloplaca subochracea* auct. belongs to *Gyalolechia*, and that two closely related species can be recognized; formerly these were treated as varieties with differently pigmented thalli, and are recombined here as *Gyalolechia marmorata* (Bagl.) Nimis & Arup, and *G. luteococcinea* (Clauzade & Cl. Roux) Cl. Roux, M. Bertrand & Arup. Finally, the name *Xanthocarpia fulva* (Harm.) Nav.-Ros. & Cl. Roux, is introduced to replace *Xanthocarpia marmorata*.

*Key words:* biodiversity, *Gyalolechia*, PCR, *Xanthocarpia*.

### Introduction

This paper clarifies the taxonomy and nomenclature of a characteristic calcicolous lichen, formerly called “*Caloplaca subochracea*”, which is widespread in coastal areas of the Mediterranean region, being known from Morocco (Egea 1996), Tunisia (Seaward 1996), Portugal (Alonso & Egea 1995; van den Boom & Giralt 2012), Spain (Llimona & Hladun 2001), southern France, including Corsica (Roux & coll. 2014), central and southern Italy, including Sicily and Sardinia (Nimis 2016), Malta (this paper), the Dalmatian coasts of Croatia (Christensen 1987), Greece (Abbott 2009), and Mediterranean Turkey

(John 1996). In spite of being easily recognizable, this lichen was often misunderstood, and has a very complicated nomenclatural history, which is detailed in the following.

The epithet *subochracea* as applied to species of *Caloplaca* s.l. has a double origin: on the one hand *Lecanora aurantiaca* var. *subochracea* Weddell (1873), on the other hand *Caloplaca subochracea* Choisy & R.G. Werner in Werner (1932: 70), which are two very different lichens, as shown by Clauzade and Roux (1973: 51-52). This was a source of confusion which has created several nomenclatural problems. Here we summarize the history of this epithet.

**Weddell (1873)** - Weddell (1873) mentions (p. 363) a new variety, *Lecanora aurantiaca* var. *subochracea* Wedd., whose protologue indicates only: “Sur les murs un peu ombragés. Thalle indiqué seulement par une tache d’un jaune très pâle”. Due to the lack of microscopic data in the description, especially on the spores, and to the absence of a *typus*, the application of this name is uncertain. Despite this, Zahlbruckner (1931) proposed the new combination *Caloplaca flavovirescens* var. *subochracea* (Wedd.) Zahlbr. (see also later, Clauzade & Roux 1973).

**Choisy & Werner (1932)** - In the Contribution à la flore cryptogamique du Maroc (V) by Werner (1932), Choisy and Werner described (p. 170) *Chrysomma subochracea* Choisy & Werner (the genus *Chrysomma* being separated from *Caloplaca*, p. 169), without basing it on *Lecanora aurantiaca* var. *subochracea* Wedd. These authors provided a diagnosis and designated a type (Morocco, Western Rif: Djebel Moussa, 650 m, at Ain Barka, limestone. leg. R.G. Werner, 28.6.1930, RAB, specimen not seen), but, as they explicitly mentioned that it is synonymous with *Caloplaca flavovirescens* var. *ochracea* (Nyl.) Zahlbr. (of uncertain application) and *Placodium ochraceum* A.L. Smith (whose current name is *Xanthocarpia ochracea*), *Chrysomma subochracea* cannot be considered as a valid new species.

**Werner (1955)** - Twenty-three years later, Werner (1955: 354-355) changed his point of view, and considered *Chrysomma subochracea* to be a synonym of *Caloplaca subochracea* (Wedd.) Werner, probably following Zahlbruckner (1951: 138) who treated *Lecanora aurantiaca* var. *subochracea* Wedd. as a synonym of *Caloplaca flavovirescens* var. *subochracea* (Wedd.) Zahlbr.

**Clauzade (1965)** - Clauzade (1965: 45) attributed the name *Caloplaca subochracea* (Wedd.) Werner to a lichen from sublittoral Provence (Massif de Marseilleveyre and around the Étang de Berre) after observing the specimens from Werner’s herbarium kept in the BCC Herbarium (communication by G. Clauzade to C. Roux, 1972).

**Clauzade & Roux (1973)** - However, as early as 1972, Clauzade and Roux strongly doubted the conspecificity between Weddell’s lichen (non-Mediterranean, colline belt) and the specimens of Werner (eu-, lower meso- and thermomediterranean belts). As they were unable to locate the type of *Lecanora aurantiaca* var. *subochracea* Wedd., Roux explored, in 1972, the locus classicus (public garden of Blossac in Poitiers, Dept. of Vienne, France) where he did not observe *Caloplaca subochracea* as understood by Werner (which was expected), but a *Caloplaca* s.l. close to *C. dolomiticola* (current genus: *Variospora*), probably corresponding to Weddell’s lichen (Clauzade & Roux, 1973: 52). Thus, Weddell’s lichen seems closer or even conspecific with *Caloplaca schaeereri* (Flörke) Zahlbr., judging from the original description by Flörke sub *Lecidea schaeereri* Flörke in Arnold (Roux & coll. 2014, 2017, 2020).

**Clauzade & Roux (1977, 1985), and Roux & coll. (2014)** - Thereafter, Clauzade & Roux (1977, 1985) named the Mediterranean lichen *Caloplaca subochracea* Werner em. Clauzade & Cl. Roux, to distinguish it from Weddell's lichen, but this did not make it a valid name designating a lichen other than Weddell's. Roux & coll. (2014) corrected its authority to *Caloplaca subochracea* (M. Choisy & Werner) Clauzade & Cl. Roux, based on *Chrysomma subochracea* Choisy & Werner (1932), but this combination was invalid (no reference to the basionym). Furthermore, Clauzade & Roux (1977: 27-31) subdivided the species into two varieties (var. *subochracea* and var. *luteococcinea*), the nominal variety being further subdivided into three forms (f. *subochracea*, f. *pallida* and f. *acrustacea*). The var. *luteococcinea* Cl. Roux (the 1977 description by Clauzade & Roux, which was invalid, was nomenclaturally validated by Roux 2003), which differs from var. *subochracea* mainly in the bright yellow-orange thallus and the ecology, will be discussed in detail later. Clauzade and Roux, and then Roux accepted the understanding of *Caloplaca subochracea* sensu Werner (non Weddell) until 2014, especially since the examination of Weddell's sample (BCC-lich) by Roux in 1986 confirmed the observations of Clauzade (see above, Clauzade 1965): the typus of *Chrysomma subochracea* corresponds to *Caloplaca subochracea* var. *subochracea* f. *subochracea* of Clauzade & Roux (1977); two other specimens are attributable, respectively to *C. subochracea* var. *subochracea* f. *pallida* [Morocco, Plage de Temara, south of Rabat (10-12 km), on agglomerated sands. Leg. Zaborsky, 16.6.1932], and f. *subochracea* (Morocco, Western Rif, Beni Hosmar mountains near Tefouare, 800 m, on limestone. Leg. Nein, 29.6.1930).

**Arup & al. (2013)** - In their major review of the *Teloschistaceae*, Arup & al. (2013), on the basis of molecular data, treated *Lecanora aurantiaca* var. *subochracea* Wedd. as *Blastenia subochracea* (Wedd.) Arup, Søchting and Frödén, but the specimen they sequenced, collected on basalt in the Azores, does not belong to *Lecanora aurantiaca* var. *subochracea* Wedd. nor to *Caloplaca subochracea* sensu Werner et auct. medit. (both of which are frankly calciphilous), but to *Blastenia festivella* (Vondrak & al. 2020: 317).

**Nimis (2016)** - Finally, Nimis (2016) was able to locate and study the typus of *Calloporisma marmoratum* Bagl. (MOD-TSB), described by Baglietto (1879) from Sardinia, and found that it did not belong to the lichen named by modern lichenologists at first *Caloplaca marmorata* (Bagl.) Jatta (Navarro-Rosinés & Hladun 1996) and then *Xanthocarpia marmorata* (Bagl.) Frödén, Arup & Søchting (Arup & al. 2013), but was identical to *Caloplaca subochracea* sensu Werner, more precisely to *C. subochracea* f. *subpallida* Clauzade & Cl. Roux. This has important consequences: we finally have a valid name, *Calloporisma marmoratum* Bagl., which can serve as a basionym for a new combination replacing *Caloplaca subochracea* auct. non (Wedd.) Werner, and also implies that another name must be found for *C. marmorata* auct. non (Bagl.) Jatta.

In this paper, the taxonomy of *Caloplaca subochracea* auct. s.lat. was studied on the basis of morphological, chemical and molecular data. The main aims are: 1) To identify the generic placement of this lichen within the *Teloschistaceae*, 2) To clarify the status of its two differently coloured varieties, 3) To solve the complicated nomenclatural matters.

## Material and methods

### *Molecular methods*

For analyses performed in this study sequences of *Teloschistaceae* subfamily *Caloplacoideae* (Arup & al. 2013) were downloaded from Genbank and complemented with 19 sequences produced for this study. Fresh material of *Caloplaca subochracea* var. *subochracea* auct. was collected in southern France and on Malta whereas material of both var. *subochracea* and of var. *luteococcinea* was collected in southern France at the type locality of the latter on the Island of Riou, Marseilles. Material from the type locality of *Callopsisma marmoratum* Bagl. could not be obtained, as this is presently a protected archaeological area where collecting rock samples is not permitted.

New sequences for this study (Table 1) were produced using direct PCR according to Arup (2006). Amplifications were made of the internal transcribed spacer regions (nrITS) and the large subunit (nrLSU) of the nuclear ribosomal RNA genes, and the small subunit of the mitochondrial ribosomal RNA gene (mrSSU). Primers for amplification were ITS1F (Gardes & Bruns 1993), ITS4 (White & al. 1990), AL1R (Döring & al. 2000), LR5 (Vilgalys & Hester 1990), mrSSU1 (Zoller & al. 1999) and mrSSU7 (Zhou & Stanosz 2001). The PCR parameters included an initial hold at 95°C for 5 min, then denaturing at 94°C for 1 min, annealing at 50°C or 54°C (mrSSU) or 53–56°C (nrITS and nrLSU) for 1 min, decreasing 1°C per cycle for the first 6 of the 39 cycles (touchdown), and extension at 72°C for 3 min. The sequencing was done by Macrogen Inc., South Korea, using the same primers as for the PCR. The two resulting strands were assembled using Geneious 11.1.5. Subsequent alignments were done in the same program and adjusted manually. Sequences have been submitted to GenBank as indicated in Table 1.

Preliminary studies of material collected in southern France strongly indicated a phylogenetic position of *Caloplaca subochracea* auct. in the genus *Gyalolechia* in subfamily *Caloplacoideae* of the family *Teloschistaceae*. In order to analyse this hypothesis in greater detail, a three-gene combined alignment of nrITS, nrLSU and mrSSU including 68 members of the subfamily *Caloplacoideae*, 2070 bases long, was prepared. In a second step, an alignment including 34 nrITS sequences of the genus *Gyalolechia*, 539 bases long, was prepared to elucidate the relationships of taxa within the species *C. subochracea* auct. and their relation to other species in the genus. In the first alignment *Xanthomendoza fallax* was used as an outgroup, in the second analysis *Leproplaca chrysodeta* was used as an external outgroup and *Caloplaca chlorina* as an internal outgroup. Introns in all the aligned genes and ambiguously aligned parts were excluded from the alignment. The alignments of the three different genes were first analysed separately to check for incongruence between genes, but none were detected. A conflict was assumed to be significant if two different relationships were both supported with posterior probabilities of 0.95 or higher.

Data were analyzed using the program MrBayes 3.2.4 (Ronquist & al. 2012). A suitable model of molecular evolution was selected using the Bayesian Information Criterion (BIC) as implemented in jModeltest ver. 2.1.4 (Guindon & Gascuel 2003; Darriba & al. 2012), evaluating only the 24 models available in MrBayes 3.2.0 (Ronquist & al. 2012). For the combined analysis the SYM+G+I model was found to be optimal for the nrITS, the GTR+G+I for nrLSU and HKY+G+I for the mrSSU data set. For the pure ITS data set GTR+G+I was found to be optimal. No molecular clock was assumed.

Tab. 1. Sequences used in any of the two analyses (see Fig.1 and Fig. 2), newly produced in bold and others downloaded from Genbank.

Species	Country, collector, collectors nr, herbarium	nrITS	nrLSU	nrSSU
<i>Blastenia catalinae</i>	Sweden, Arup L06075, LD	FJ866792		KT291532
<i>Blastenia crenularia</i>	Iceland, Søchting US7523, C	KC179415		KC179492
<i>Bryoplaça jungermanniae</i>	Greenland, Søchting US 10451, C	KC179420		MT952895
<i>Bryoplaça tetraspora</i>	Antarctica, Søchting US7979, C (ITS); Greenland, Søchting US10480, C (LSU, SSU)	KC179422		MT952896
<i>Caloplaça cerina</i>	Svalbard, Elvebakk 03-109, TROM	KC179425		
<i>Eiliffåhlla dahlii</i>	Australia, Kärnefelt 20043101, LD	KJ021318		KJ021279
<i>Eiliffåhlla wirthii</i>	Australia, Wirth et al. 05-10-2011, STU	KJ021320		KJH021255
<i>Franswisia kilcundaensis</i>	Australia, Kärnefelt 20047101, LD	KJ021327		KJ021287
<i>Franswisia bastowii</i>	Australia, Kärnefelt 994301, LD	KJ021324		KJ021284
<i>Gyalolechia allochroa</i>	South Korea, Joshi et al. JV7987, CBFS	HQ415800		KC179529
<i>Gyalolechia arizonica</i>	USA, Arizona, Nash 38931, C	KC179433		KC179530
<i>Gyalolechia aurea</i>	Arup L97493, LD (ITS, SSU)	KC179434		
<i>Gyalolechia bassiae</i>	Austria, 1993, Poelt & Grube, GZU (LSU)	KC179435		
<i>Gyalolechia bracteata</i>	Mauritius, Søchting 9748, C	AF277668		
	Austria, Lutzoni 96.8.30-19, C	MT952926		
	Canary Islands, Étayo & Rebolé 17566, C	KC179436		MT952927
	Australia, Kondratyuk 20441, LD	KJ021334		KJ011293
	Unknown	AF277668		
	Israel, 2005, Garty, C	KC179438		
	China, Vondrák JV12412, PRA	KU360122		
	Estonia, Søchting US10127, C	KC179439		KC179531
	Russia, Søchting US8648, C	AF353966		KC179532
	Unknown	AY233221		
	Spain, Søchting US7306, C (ITS); Sweden, Arup L06206, LD (LSU)	KC179440		KC179533
	South Korea, Hur 080515, KoLRI	KJ021231		
" <i>Gyalolechia</i> " <i>gordjevii</i>	Spain, Søchting US9653, C	KC179441		KC179534
<i>Gyalolechia gomeraana</i>	Russia, Søchting 8634, C	KC179442		
<i>Gyalolechia lenae</i>	France, Bertrand & Roux 10, LD	OQ235059		OQ248479
<i>Gyalolechia luteococcinea 1</i>	France, Bertrand & Roux 10, LD	OQ235060		
<i>Gyalolechia luteococcinea 2</i>	France, Bertrand & Roux 10, LD	OQ235061		
<i>Gyalolechia luteococcinea 3</i>	France, Bertrand & Roux 9, LD	OQ235062		
<i>Gyalolechia marmorata 1</i>	France, Bertrand & Roux 9, LD	OQ235063		
<i>Gyalolechia marmorata 2</i>	France, Bertrand & Roux 9, LD	OQ235064		
<i>Gyalolechia marmorata 3</i>	France, Bertrand & Roux 9, LD	OQ235065		
<i>Gyalolechia marmorata 4</i>	Malta, Rabat 1 Jan 2020, Fiorentino, TSB	OQ235066		
<i>Gyalolechia marmorata 5</i>	France, Arup L19416, LD	LC490369		OQ236406
" <i>Gyalolechia</i> " <i>multicolor</i>	Japan, Harada 32273, CBM	AF279884		
<i>Gyalolechia subbracteata</i>	Unknown	KC179443		
<i>Gyalolechia oxneri</i>	Russia, 17 sept 1989, Kondratyuk s.n., LD isotype	KC179444		
<i>Gyalolechia persimilis</i>	USA, Texas, Wetmore 73334, C	AF279880		
<i>Gyalolechia pruinosia</i>	Unknown			

Tab. 1. continued.

<i>Gyalolechia</i> sp.		OO2:35067	KC179201	KC179535
<i>Gyalolechia stantoni</i>	Teneriffe, Klepsland JK14-1220, priv. herb.	KC179445	KC179202	KC179536
<i>Gyalolechia stipitata</i>	USA, California, Wetmore 73334, C	KC179446		
<i>Gyalolechia xanthostigmaidea</i>	Mexico, Søchting US9917, C	KC179447		
<i>"Gyalolechia" yeosuensis</i>	Denmark, Greenland, Søchting 10461, C	KJ021235		
<i>Huneckia pallini</i>	South Korea, Jayalal et al. 120360, KoLRI	KJ021336	KJ021265	KJ021296
<i>Jasonhuria bogliana</i>	USA, Kansas, Morse 14464, LD	MT952903	MT952903	MT952929
<i>Lacrima epiphora</i>	Galapagos, Aprroot 63246, CDS (ITS); Miranda 962, CDS (LSU, S SSU)	KT220205	KT220205	KT220214
<i>Lacrima galapagoensis</i>	South Korea, KoLRI 120454	MT952904	MT952904	MT952930
<i>Leproplaca chrysoidea</i>	Panama, van den Boom 43698, priv. herb.	MT952905	MT952905	MT952931
<i>Leproplaca obliterans</i>	Galapagos, Bungartz 4091, CDS (ITS); Ertz 22855, CDS (LSU, SSU)	KC179206	KC179206	MT952933
<i>Leproplaca xantholyta</i>	Sweden, Arup L07107, LD (ITS, LSU); Sweden, Arup L13261, LD (SSU)	KC179207	KC179207	KC179541
<i>Loekaesia austrocoreana</i>	Sweden, Arup L02331, LD (ITS, SSU), Norway Arup L03472, LD (LSU)	KC179208	KC179208	KC179542
<i>Loekaesia yuchiorum</i>	Austria, Arup L97278, LD (ITS), Spain, Søchting US9675, C (LSU, SSU)	KT220210	KT220210	KT220219
<i>Oleghlumia demissa</i>	South Korea, Jayalal et al. 120523, KoLRI	OO2:36407	OO2:36407	OO2:48482
<i>Kuettlingeria erythrocarpa</i>	USA, South Carolina, Lendemer 22026, LD	KC179172	KC179172	KC179505
<i>Kuettlingeria teicholytha</i>	Italy, Arup L07109, LD	KC179173	KC179173	KC179506
<i>Marchantiana asserrigena</i>	Denmark, US11195, C (ITS, LSU); Denmark, US9772, C (SSU)	KC179176	KC179176	KC179510
<i>Marchantiana queenslandica</i>	Scotland, Arup L10184, LD	MT952907	MT952907	MT952934
<i>Oceanoplaca isidifosa</i>	Australia, Kalb 27764, CANB	MT952908	MT952908	-
<i>Obscuroplaca campitida</i>	Cape Verde, van den Boom 36365, LD	MT952909	MT952909	MT952935
<i>Obscuroplaca ochrolechiooides</i>	Galapagos, Anderssen, LAM8-1, C	MT952913	MT952913	MT952938
<i>Pyrenodesmia alocliza</i>	USA, Morse 14420, LD	MT952918	MT952918	MT952942
<i>Pyrenodesmia chalybaea</i>	Australia, Kalb & Rogers 18982, CANB (ITS);	MT952919	MT952919	-
<i>Pyrenodesmia variabilis</i>	Sweden, Arup L10185, LD (LSU, SSU)	MT952920	MT952920	MT952944
<i>Rufoplaca scotoplica</i>	Austria, Søchting US9351, C	KC179571	KC179571	KC179571
<i>Sanguinodiscus aractinus</i>	Austria, Arup s.n., LD (ITS); Sweden, Arup L03134, LD (LSU, SSU)	KC179234	KC179234	KC179572
<i>Sanguinodiscus haematites</i>	Sweden, Arup L10032, LD	KC179235	KC179235	KC179573
<i>Seiophora blumii</i>	Norway, Arup L08171, LD	KC179237	KC179237	KC179575
<i>Seiophora californica</i>	Czech Republic, Vondrák 6702, PRA	OO2:36408	OO2:36408	MH100773
<i>Seiophora lacunosa</i>	Ukraine, Vondrák 7278, PRA	MH100789	MH100789	MH100756
<i>Seiophora cf. mediterranea</i>	Iran, Haji Moniri A1, 4, KW	KT456234	KT456234	KT456249
<i>Seiophora scorigera</i>	Mexico, Gava 03.04.10-9 & Lutzoni, DUKE	KT291564	KT291564	KT291521
<i>Suocoplaca diplicia</i>	Kazakhstan, Moberg & Nordin K18:04, LD	KC179582	KC179582	KC179582
<i>Usnochroma carphinea</i>	Ukraine, Khodosovtsev s.n. KW 70478	KT220222	KT220222	KT220222
<i>Usnochroma scoriophila</i>	Lanzarote, Snogerup, S. & B. 17201, LD	KC179583	KC179244	KC179583
<i>Variospora aurantia</i>	Galapagos, Ertz 11610, CDS	MT952945	MT952945	MT952945
<i>Variospora dolomitica</i>	France, 1998, Roux, C	KC179259	KC179259	KC179598
<i>Variospora flavescens</i>	Teneriffe, 1995, Gomez-Bolea, C	KC179260	KC179260	KC179599
<i>Variospora glomerata</i>	Spain, 1998, Ilmona, C (ITS, SSU); Italy, 2006, Lange, C (LSU)	KC179261	KC179261	KC179600
<i>Xanthomendoza fallax</i>	Spain, Thell SP0514, LD	KC179262	KC179262	KC179601
<i>Yoshimuria galbana</i>	Denmark, US9601, C (ITS); Sweden, Arup L03060, LD (LSU, SSU)	KC179263	KC179263	KC179602
<i>Yoshimuria spodioplaça</i>	Sweden, Arup L03119, LD	KC179264	KC179264	KC179603
	USA, Alaska, Gava et al, DUKE	JQ301580	JQ301580	JQ301522
	South Korea, Arup L15370, LD (ITS); Arup 15500, LD (LSU, SSU)	MT967482	MT967482	MT952946
	South Korea, Wang & Ryu 110364, KoLRI (ITS, SSU); Arup L15575, LD (LSU)	KJ021249	KJ021249	KJ023194



Three parallel runs with 2,000,000 generations starting with a random tree and employing 6 simultaneous chains was executed, of which 5 were incrementally heated with a temperature of 0.10. Analyses were diagnosed every 1000 generations in the last 50% of the tree sample and automatically halted when convergence was reached. Convergence was defined as a standard deviation of splits (of frequency 0.1) between runs below 0.01. Every 1000th tree was sampled. A majority-rule consensus tree was constructed from the post-burn-in tree samples. The consensus trees was visualized using FigTree 1.4.4 and re-drawn in Adobe Illustrator.

### **Secondary chemistry**

Secondary metabolites of the fresh material collected in southern France were analysed with high performance liquid chromatography (HPLC), following the standard procedure outlined in Søchting (1997). Thallus and apothecia were analysed separately. The HPLC used here does not allow direct quantification, but proportions between compounds as measured at 270 nm. When only one compound is present it will result in 100%, even if only present in small amount. Comparison between species can only be made if exactly the same sample size has been used.

## **Results**

### ***The type of Callopisma marmoratum Bagl.***

*Callopisma marmoratum* Bagl. was described from Sardinia by Baglietto (1879), on the basis of material collected by G.B. Canepa and P. Gennari at Piazza d'Armi, in the surroundings of Cagliari, on calcareous rocks. Three samples of this species, all belonging to the same collection from the type locality, were found in MOD by Nimis in 1997 accidentally, as they were displaced from the main folders containing species of *Teloschistaceae* in the Herbarium Baglietto. Two of them, distributed as exsiccata in Erbario Crittogamico Italiano, ser. II nr. 67, can be considered as isotypes, the third is annotated by Baglietto himself and is the holotype. The type locality is presently heavily urbanised, but is adjacent to a large semi-natural area with many calcareous outcrops where the lichen is still present, but cannot be collected, as the whole area hosts a necropolis, and is therefore protected.

Brief description of the type material: Thallus crustose, thinly episubstratic, continuous to finely rimose-areolate, pure white to pale yellow near the apothecia, delimited by a thin, black prothalline line. Apothecia biatorine, at first immersed, then sessile, rounded, 0.2-0.8 mm across, with a rusty red to dark orange-red, at first flat, then convex, epruinose disc, and a somewhat paler, smooth, at first well-visible, finally often excluded proper margin (most apothecia of the type appear to be grazed by gasteropods). Epithecium brownish orange, K+ red; hymenium colourless, 40-60(-70)  $\mu\text{m}$  high; paraphyses simple or forked in upper part, c. 1-1.5  $\mu\text{m}$  thick at base, the apical cells (2-)2.5-3.5  $\mu\text{m}$  wide; hypothecium colourless. Asci 8-spored, clavate, *Teloschistes*-type. Ascospores 1-septate, polarilocular, hyaline, ellipsoid, (9-)10-13(-14)  $\times$  (3-)5-6(-10)  $\mu\text{m}$ , the central thickening 3-5  $\mu\text{m}$ . Spot tests: thallus K- or K+ faintly red in the pigmented parts, C-, KC-, P-; apothecia K+ red. This description is well congruent with the original description by Baglietto (1879), and corresponds to *C. subochracea* var. *subochracea* f. *pallida* Clauzade & Cl. Roux,

described from France (holotypus in herb. G. Clauzade, MARSSJ: Aude, Narbonne, montagne de la Clape, 1974/08/05); in French material the apical cells of paraphyses are slightly wider (3–4.5  $\mu\text{m}$ ).

### ***Molecular phylogeny***

The results of the molecular studies are presented in two phylogenetic trees (Figs 1, 2). The three-gene tree (Fig. 1) constructed from nrITS, nrLSU and mrSSU sequences show the position of *Caloplaca subochracea* auct. within the subfamily *Caloplacoideae*, where it forms a fully supported clade together with the rest of the genus *Gyalolechia* sensu Arup & al. (2013). The two analysed varieties, one with a white the other with a yellow thallus (in the tree as species), form a clade, also fully supported, but clearly separated on two separate branches indicating their independentness. The second tree (Fig. 2), based on nrITS sequences of *Gyalolechia*, further supports the idea of two distinct species, since they form two separate clades with good support (1 and 0.98 respectively). They will be henceforth named *Gyalolechia marmorata* and *G. luteococcinea* (Figs 3a, 3b, see last section). In addition a third taxon, *Gyalolechia* sp., collected on *Euphorbia balsamifera* on the island of Tenerife, sits in between the two species, differing significantly from *G. luteococcinea* in 17 bases.

### ***Status of Caloplaca subochracea var. luteococcinea***

In his monograph on the lichen vegetation of the islands near Marseille, Clauzade (1970) distinguished from *Caloplaca subochracea* sensu Werner, a *Caloplaca* with a bright yellow thallus and red apothecia which he named *C. africana* (Flagey) Clauzade (p. 32), a taxon which does not appear neither in Index Fungorum nor in Mycobank, although it has been validly combined (see later). However, in 1973 the examination by Clauzade and Roux of an isotypus of Flagey's lichen (PC) showed that it did not correspond to the lichen from Marseilles, neither by gross morphology nor by microscopy, but to a "form of *C. dolomiticola* with an orange thallus, red apothecia and almost globose spores (13–14  $\times$  9–11  $\mu\text{m}$ )" (Clauzade & Roux 1977: 28), therefore to a representative of the current genus *Variospora*. Furthermore, the comparison between *C. subochracea* sensu Werner and *C. africana* sensu Clauzade has shown (Clauzade & Roux 1977: 28–29) that they are practically identical in their microscopic characters, that the apothecia of the former can sometimes be red (those of the latter are always so), that they are distinguished essentially by the colour of the thallus (bright yellow in *C. africana* sensu Clauzade, from greyish or chalky white to pale yellow in *C. subochracea* sensu Werner), and that transitional forms can exceptionally be observed. Consequently, Clauzade & Roux (1977) described the lichen from Marseilles as *C. subochracea* var. *luteococcinea* Clauzade & Cl. Roux, a name which was later validated nomenclaturally by Roux (2003). Forty-five years later, taxonomic ideas and methods having evolved considerably, M. Bertrand and C. Roux thought it was essential to compare the DNA of *C. subochracea* sensu Werner with that of *C. subochracea* var. *luteococcinea* to test whether the latter was an independent species rather than a variety. Thanks to the collaboration of the Calanques National Park, on 22/04/2022 they visited the island of Riou (Marseille), which is the locus classicus of *C. subochracea* var. *luteococcinea* and also hosts *C. subochracea* var. *subochracea* sensu Werner, where they collected specimens of both lichens (including topotypes of var. *luteococcinea*). The



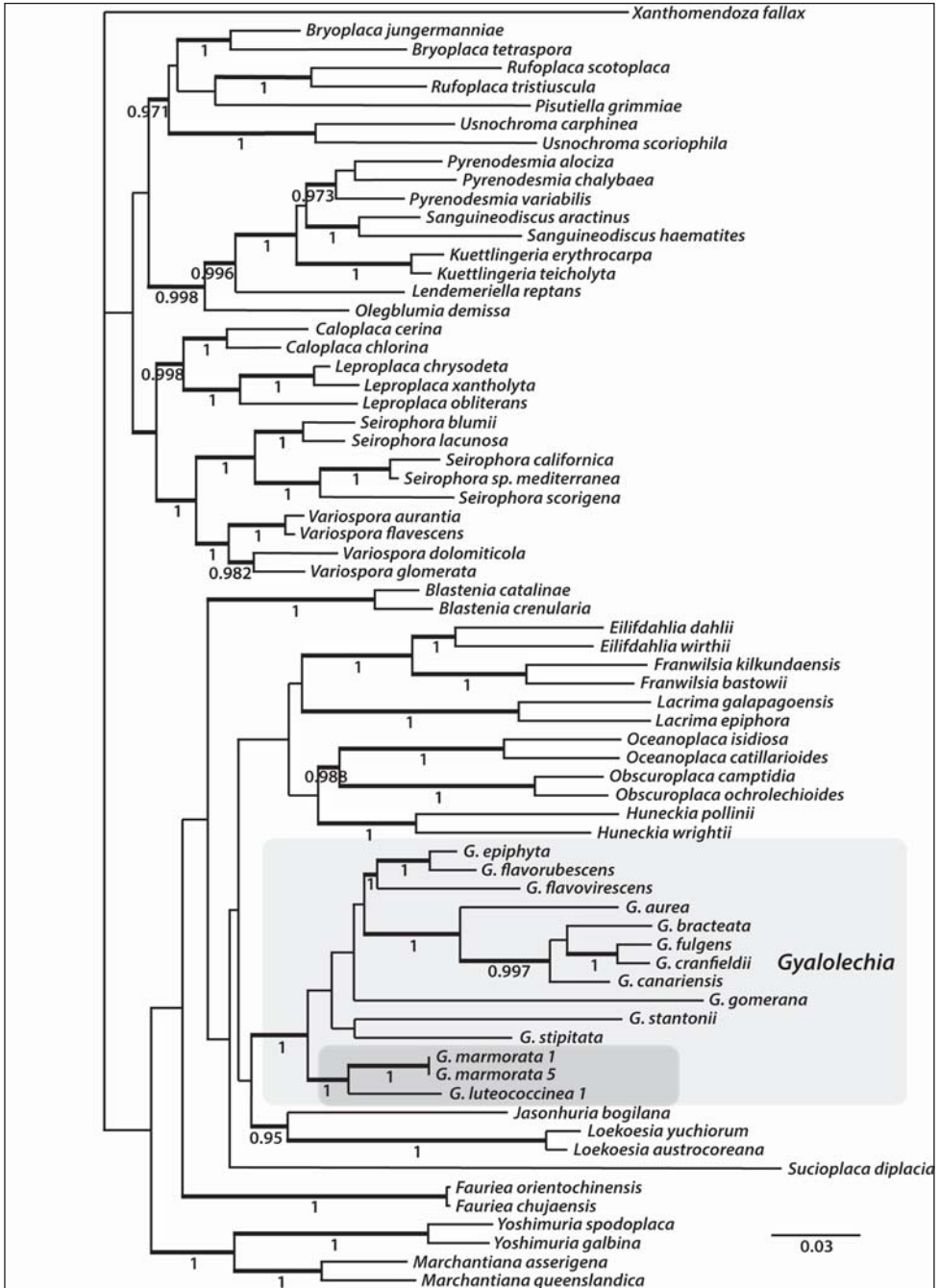


Fig. 1. Majority-rule consensus tree based on a Bayesian MCMC analysis of a combined data set of the ITS, LSU and SSU genes showing the position of *Gyalolechia marmorata* and *G. luteococcinea*. Branches with posterior probabilities higher or equal to 0.95 are shown in bold.

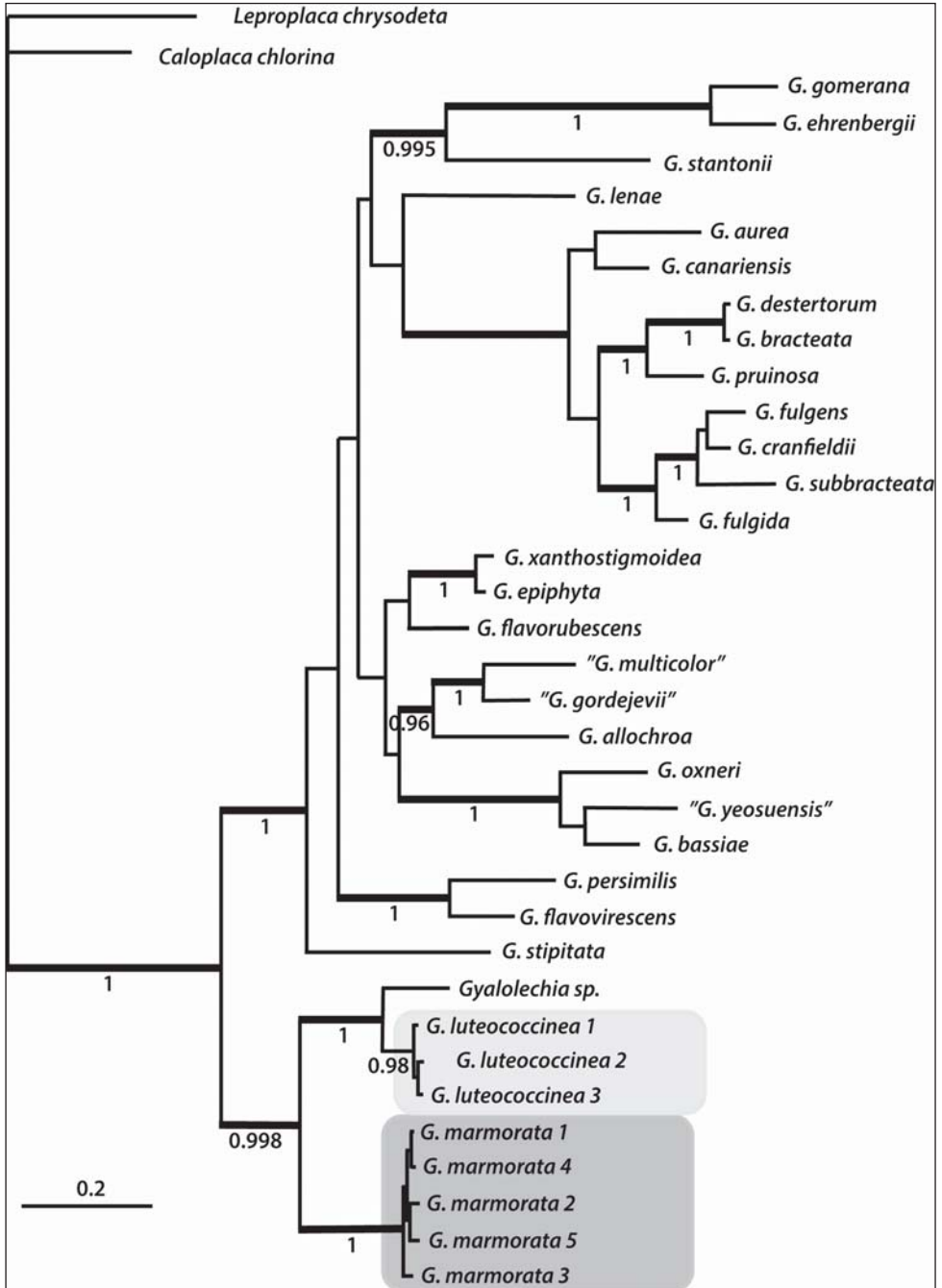


Fig. 2. Majority-rule consensus tree based on a Bayesian MCMC analysis of ITS data of the genus *Gyalolechia* showing the position of *Gyalolechia marmorata* and *G. luteococcinea* in the genus. Branches with posterior probabilities higher or equal to 0.95 are shown in bold.



Fig. 3. a) *Gyalolechia marmorata*; b) *Gyalolechia luteococcinea*, topotype. Photos by M. Bertrand.

results of our molecular analysis showed that both taxa are related, belonging to *Gyalolechia*, but that they show enough differences to be distinguished from each other at species level. *Gyalolechia luteococcinea* (Fig. 1b), which often occurs together with *G. marmorata*, is known from Morocco (Egea 1996), Portugal (Alonso & Egea 1995), Spain (Llimona & Hladun 2001), southern France, including Corsica (Roux & Coll. 2014), and central and southern Italy, including Sicily (see Appendix).

### **Secondary chemistry**

The analysis of the secondary chemistry of *G. marmorata* and *G. luteococcinea* (Tab. 2) shows that the apothecia of the two species have a very similar chemical pattern, with parietin and emodin as major substances, and citreorosein, emodin, fallacinal and parietinic acid as minor substances. The thalli of both species contain only fragilin, which, however, is 10-15 times more concentrated in the thalli of *G. luteococcinea* than in those of *G. marmorata*. This is the reason for the stronger thallus pigmentation in the former species.

### **A new name for *Xanthocarpia marmorata* auct.**

Nimis & Poelt (1987) tentatively attributed the epithet *Caloplaca marmorata* (Bagl.) Jatta to a calcicolous lichen with rusty-red apothecia, whose spores have a very short “septum” (thus belonging to *Xanthocarpia*), based on the analysis of a sample from MOD, labelled *Blastenia ferruginea* var. *carolitana* Bagl., a name which was never published, collected in the same locality as *Callopsisma marmoratum* Bagl., the type of the latter being not available at that time. Its subsequent discovery showed that the spores of *C. marmoratum* have a long septum, which was also depicted in a drawing by Baglietto (1879), and that the type material has nothing to do with a *Xanthocarpia*. According to Navarro-Rosinés & Hladun (1996), among the synonyms of *X. marmorata* sensu auct., the only one at the specific level, *C. flageyana* (Flagey) Zahlbr., is of uncertain application because, being parasitized by a *Tremella*, it has less brightly colored apothecia and poorly developed spores. Of the three described forms attributable to *X. marmorata* auct., the type of *Caloplaca lactea* f. *rubra* (B. de Lesd.) Zahlbr. (basionym *Gyalolechia lactea* f. *rubra* B.

Tab. 2. Anthraquinones (%) of *Gyalolechia luteococcinea* and *G. marmorata* (th: thallus; apoth: apothecia).

	<i>G. luteococcinea</i> apoth.	<i>G. luteococcinea</i> th.	<i>G. marmorata</i> apoth.	<i>G. marmorata</i> th.
citreorosein	1			
emodinal	5			
emodinic acid	2		2	
fallacinal	4		6	
parietinic acid	2		5	
emodin	45		19	
parietin	41		68	
fragilin		100		100

de Lesd., 1911) has probably disappeared and is therefore not usable, while *Caloplaca lactea* var. *laetior* Steiner (1921) and *Caloplaca lactea* var. *fulva* (Harm.) Zahlbr. (basionym: *Lecanora lactea* f. *fulva* Harm., 1913) are both represented by a type deposited in a museum. *Lecanora lactea* f. *fulva* Harm., which pre-dates *Caloplaca lactea* var. *laetior*, could therefore be recombined in *Xanthocarpia* (see next section).

### Taxonomic and nomenclatural consequences

The previous considerations have several taxonomic and nomenclatural consequences, which are summarized in the following.

***Blastenia festivella*** (Nyl.) Vondrák, J. Syst. Evol., 58(3): 295-330 (2020)

Basion. *Lecanora ferruginea* var. *festivella* Nyl., Flora, Regensburg, 56: 197 (1873).

– *Blastenia subochracea* sensu Arup & al. 2013, in Arup, Søchting & Frödén, Nordic J. Bot., 31(1): 68 (2013) [non *Caloplaca subochracea* (Wedd.) Werner].

***Caloplaca subochracea*** (Wedd.) Werner, Bull. Soc. bot. Fr., 102: 354 (1955)

Basion. *Lecanora aurantiaca* var. *subochracea* Wedd., Mém. Soc. imp. Sci. nat. Cherbourg 17: 363 (1873) [no type material nor a sufficient description are available, therefore a name of uncertain application, as well as all names based on this taxon; perhaps a synonym of, or related to *Variospora schaeereri* (Flörke) Cl. Roux, Bull. Ass. fr. Lichénol., 47(1): 30, Basion.: *Lecidea schaeereri* Flörke in Arnold, Flora (Regensburg), 64: 312 (1881)].

≡ *Caloplaca erythrella* var. *subochracea* (Wedd.) H. Olivier, Mém. Soc. nation. Sci. nat. Cherbourg, 37: 114 (1909).

≡ *Caloplaca flavovirescens* var. *subochracea* (Wedd.) Zahlbr., Cat. Lich. Univers., 7: 138 (1930) [1931].

***Caloplaca subochracea*** (M. Choisy & Werner) Clauzade & Cl. Roux in Catalogue des lichens de France: 253 (2014), comb. inval. (no reference to the basionym).

***Chrysomma subochracea*** M. Choisy & Werner, Cavanillesia, 5(5): 170 (1932) [name of uncertain application].

***Gyalolechia luteococcinea*** (Cl. Roux) Cl. Roux, M. Bertrand & Arup comb. nov. MB 847180

Basion. *Caloplaca subochracea* var. *luteococcinea* Cl. Roux, Bull. Soc. Linn. Provence, 54: 120 (2003) (nomenclatural validation).

≡ *Caloplaca subochracea* var. *luteococcinea* Clauzade & Cl. Roux, Bull. Soc. Linn. Provence, 30: 27-31 (1977), comb. inval.

– *Caloplaca africana* sensu Clauzade 1970 non sensu (Flagey) Clauzade.

***Gyalolechia marmorata*** (Bagl.) Nimis & Arup comb. nov.. MB 847181

Basion. *Calloposma marmoratum* Bagl., N. Giorn. Bot. Ital., 11: 84 (1879).

≡ *Caloplaca marmorata* (Bagl.) Jatta, Sylloge Lich. Ital.: 251 (1900), non sensu auct.

***Xanthocarpia fulva*** (Harm.) Nav.-Ros. & Roux comb. nov. MB 847183

Basion. *Lecanora lactea* f. *fulva* Harm., Lich. Fr., 5: 863 (1913).

– *Caloplaca marmorata* auct. non (Bagl.) Jatta.

– *Xanthocarpia marmorata* auct. non (Bagl.) Frödén, Arup & Søchting.

***Variospora africana*** (Flagey) Cl. Roux comb. nov. MB 847182

Basion. *Caloplaca aurantiaca* var. *africana* Flagey Catalogue des lichens de l'Algérie: 32 (1896).

≡ *Caloplaca africana* (Flagey) Clauzade, Portugaliae Acta Biologica, sér. B, 11(1-2): 32 (1970).

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Addresses of the authors:

Ulf Arup<sup>1</sup>, Michel Bertrand<sup>2</sup>, Pere Navarro-Rosinés<sup>3</sup>, Pier Luigi Nimis<sup>4\*</sup>, Claude Roux<sup>5</sup> & Ulrik Söchting<sup>6</sup>,

<sup>1</sup>Botanical Museum, Lund University, Box 117, SE-22100 Lund.

<sup>2</sup>La Grande Bastide, FR-84750, Viens.

<sup>3</sup>Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals (BEECA), Secció de Botànica i Micologia. Institut de Recerca de la Biodiversitat (IRBio). Facultat de Biologia. Universitat de Barcelona (UB), Diagonal, 643. ES-08028 Barcelona

<sup>4</sup>Dept. of Life Sciences, University of Trieste, Via Giorgieri 10, I-34127 Trieste.

<sup>5</sup>Chemin des Vignes-Vieilles 390, FR-84120 Mirabeu.

<sup>6</sup>Dept. of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen.

\*Corresponding author: [nimis@units.it](mailto:nimis@units.it)

## Appendix – Specimina visa

### Material used for the molecular and chemical analyses

#### *Gyalolechia marmorata*:

– France, Provence, Bouches-du-Rhône, Marseille, île de Riou, 200 m à l'E de la plage du Mounestéru, alt. 60 m, paroi de calcaire urgonien (barrémien) exposée au N, pente de 80°, 2022/04/22, leg. et det. M. Bertrand et C. Roux, herb. M. Bertrand. – France, Provence, Bouches-du-Rhône, La Couronne, E side of the cove Anse du Verdon, on W-exposed limestone rocks, elev. 5 m, 2019/05/20, leg U. Arup L19416, herb. LD n° 2067433. – Malta, Ghemmieri Rabat, on limestone, 2020/01, J. Fiorentino TSB

#### *Gyalolechia luteococcinea*:

– France, Provence, Bouches-du-Rhône, Marseille, île de Riou, 200 m à l'E de la plage du Mounestéru, alt. 60 m, paroi de calcaire urgonien (barrémien) exposée au N, pente de 80°, 2022/04/22, leg. M. Bertrand et C. Roux, det. et herb. C. Roux, (MARSSJ), herb. M. Bertrand n° 6793, topotypus.

#### Localities of *Gyalolechia luteococcinea* (MARSSJ, Herb. M. Bouly de Ledain, G. Clauzade and C. Roux)

– France, Bouches-du-Rhône, Marseille, île de Riou, vallon du Mounestéru, alt. 50 m, sur bloc éboulé de calcaire urgonien, 1969/09/28, leg. et det. G. Clauzade et Cl. Roux, herb. G. Clauzade (MARSSJ) sans n°, **holotypus**. – France, Bouches-du-Rhône, Marseille, île de Riou, 200 m à l'E de la plage du Mounestéru, alt. 60 m, paroi de calcaire urgonien (barrémien) exposée au N, pente de 80°, 2022/04/22, leg. M. Bertrand et C. Roux, det. et herb. C. Roux (MARSSJ), **topotypus**. – *ibid.*, herb. M. Bertrand, n° 6793. – France, Corse-du-Sud, Bonifacio, E de Ciappili, inter Piantarella k Sperone, alt. 20 m, orient. gen. N, orient. lok. N, deklivo 80°, sur krutažo el kalka grejso (molaso), 1985/04/04, leg., det. et herb. C. Roux, n° 20121 (MARSSJ). – Spain, NE de Alicante, Calpe, Peñon de Ifach, alt. 180 m, base de la grande paroi N de calcaire compact, 1977/08/05, leg. et det. C. Roux, herb. G. Clauzade (MARSSJ), without nr.°

#### Localities of *Gyalolechia luteococcinea* from Italy (TSB, SI)

– Italy. Toscana. Prov. Livorno. Parco Nazionale dell'Arcipelago Toscano, Isola di Pianosa, along the coast, 20 m, on calcareous rocks, 20 m. 42.583987 10.074490 Leg. L. Muggia & M. Tretiach 2005. TSB 36720. – Italy. Puglia. Prov. Lecce. Otranto Torre della Serpe, on calcareous rocks, 5 m. 40.141823 18.505756. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22373. – Italy. Puglia. Prov. Lecce. Santa Cesarea Terme, on calcareous rocks, 50 m. 40.038394 18.459586. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22594. – Italy. Puglia, Prov. Lecce, Between San Gagliano del Capo and Marina di Novaglie, 60 m, on calcareous rocks. 39.851354 18.389317. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22609. – Italy. Puglia. Prov. Taranto. Martina Franca, Bosco delle Pianelle, 400 m, on calcareous rocks. 40.6418 17.2121. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22256, 22383. – Italy. Sicilia. Prov. Trapani. Island of Marettimo, 150 m, on calcareous rocks. 37.97289637 12.05904524 Leg. P.L. Nimis & M. Tretiach 1991 TSB 15213, 15233. – Italy. Sicilia. Prov. Trapani. Monte Passo del Lupo, Riserva dello Zingaro, 650 m, on calcareous rocks. 38.121691 12.754294. Leg. S. Loppi 1995. SI 135.

**Localities of *Gyalolechia marmorata* (MARSSJ and Herb. Bertrand)***Typical form (thallus white):*

– France, Aude, Narbonne, “montagne” de la Clape, près de la ligne de faîte, au SW de la route D168, alt. 140 m, sur bloc de calcaire barrémien, 1975/08/05, leg., det. et herb. G. Clauzade (MARSSJ) sans n°, holotypus de *Caloplaca subochracea* f. *pallida* Clauzade et Cl. Roux. – France, Corse-du-Sud, Bonifacio, inter Bonifacio k Pertusato, chemin du Pertusato, inter malnova baterio k malnova fuorteto, apud la vojo, alt. 20 m, orient. gen. U, orient. lok. -, deklivo 30°, sur horizontala roksurfaco el kalka grejso (molaso), 1985/04/04, leg., det. et herb. C. Roux, n° 20119, deux spécimens (MARSSJ). – Italy, Liguria occidentale, Verezzi, 1955/04/13, leg. C. Sbarbaro, det. G. Clauzade, herb. M. Bouly de Lesdain (MARSSJ).

*Form with a pale yellow thallus:*

– France, Bouches-du-Rhône, Châteauneuf-les-Martigues, 200 m au S des réservoirs d’eau, alt. 100 m, sur paroi de calcaire urgonien exposée au N, 1964/03/26, leg., det. et herb. G. Clauzade, sans n° (MARSSJ). – France, Bouches-du-Rhône, Martigues, plaine de Bonnieux, à environ 300 m du bord de la mer, dans un *Brachypodium ramosi*, sur grosses pierres (calcaire burdigalien), 1970/06/30, leg., det. et herb. G. Clauzade, sans n° (MARSSJ). – France, Corse-du-Sud, Bonifacio, tuj E de la urbo k N de la haveno, klifo antaŭ la plataĵo, alt. 20 m, orient. ĝen. N, orient. lok. N, deklivo 90°, sur krutaĵo el kalka grejso (molaso), 1985/04/04, MARSSJ, leg., det. et herb. C. Roux, n° 20118. – France, Provence, Bouches-du-Rhône, Fos-sur-Mer, Mourre Poussiou, sur molasse finement poreuse d’une barre rocheuse sommitale orientée ouest, alt. 20 m. Leg. et det. M. Bertrand 2012. Herb. M. Bertrand n° 2889.

**Localities of *Gyalolechia marmorata* (TSB)**

– Italy. Puglia. Prov. Lecce. Gallipoli, Loc. Torre del Pizzo, 2 m, on calcareous rocks, 39.996050 17.999460. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22345. – Italy. Puglia. Prov. Taranto. Martina Franca, Bosco delle Pianelle, 400 m, on calcareous rocks, 40.6418 17.2121. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22283. – Italy. Puglia. Prov. Lecce, Marina di Novaglie, Adriatic coast, 60 m, 39.868207 18.392378. Leg. P.L. Nimis & M. Tretiach 1996. TSB 22632. – Italy. Puglia. Prov. Foggia. Tremiti Islands, Isola di S. Nicola. 30 m, 42.124018 15.511480. Leg. P.L. Nimis & M. Tretiach 1997. TSB 26273. – Italy. Sardinia. Prov. Cagliari. Capo S. Elia, 50 m, on calcareous rocks, 39.18277122 9.160715246. Leg. C. Roux & M. Tretiach 1997. TSB 13311. – Italy. Sardinia. Prov. Sassari. Nuraghe Speranza, 15 m, on calcareous rocks, 40.78038 8.396510699. Leg. P.L. Nimis & M. Tretiach 1989. TSB 11813. – Italy. Sardinia. Prov. Oristano. Sinis Peninsula, Archaeological Area of Tharros, 6 m, on soft calcareous rocks, 39.8735675 8.441675699. Leg. P. L. Nimis, C. Roux, M. Tretiach & A. Vězda 1989. TSB 13040. – Italy. Sardinia. Prov. Nuoro. Punta Corongius above Jerzu, 850 m, on calcareous rocks 39.76433906 9.486606044. Leg. P. L. Nimis, C. Roux, M. Tretiach & A. Vězda 1989. TSB 13174. – Italy. Sicily. Prov. Trapani. Egadi Islands, Island of Marettimo, 500 m, on calcareous rocks, 37.97323388 12.05551616. Leg. P.L. Nimis 1991. TSB 20187. – Italy. Sicily. Prov. Trapani. Village of Erice, 730 m, walls of the castle, on calcareous rocks, 38.04098992 12.58591438. Leg. M. Tretiach 1991. TSB 16273. – Italy. Sicily. Prov. Trapani. Riserva Naturale dello Zingaro, 200 m, on calcareous rocks, 38.1040866 12.81476965. Leg. P.L. Nimis & M. Tretiach 1995. TSB 21521. – Italy. Sicily. Prov. Agrigento. Pelagie Islands, Island of Lampedusa, Loc. Punta Parise, 20 m, on calcareous rocks, 35.51374536 12.57716169. Leg. P.L. Nimis & M. Tretiach 1992. TSB 17183, 17241. – Italy. Sicily. Prov. Agrigento. Pelagie Islands, Island of Linosa, 20 m, 35.86246842 12.86495604. Leg. P.L. Nimis & M. Tretiach 1992. TSB 17197. – Portugal. Estremadura. Cabo Espichel, 60 m, on calcareous rocks. Leg. P.L. Nimis 1996. TSB 25386.

